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UTERINE MACROPHAGES IN THE MOUSE AND THEIR RELATION TO INVOLUTION¹

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TWO TEXT FIGURES AND EIGHT PLATES (FIFTY-TWO FIGURES)

A study of the uterus in the parous mouse revealed that the placental sites disappear very rapidly. By the end of the second week post-partum each site was represented macroscopically by a brown area at the base of the mesentery. Microscopically each area was found to consist of masses of unusually large cells whose color was due to the presence in the cytosomes of dark brown granules of various shapes and sizes. These granules were identified as consisting of the iron-containing substance hemosiderin, and the cells were interpreted as belonging to the migratory group of reticulo-endothelial cells. They will be spoken of in the following pages as macrophages. Each aggregation of hemosiderin-laden macrophages constitutes what may be termed a mesometrial hemosiderotic area.

These hemosiderotic areas have been noted by other workers, and various explanations have been offered concerning their origin and function. In a recent paper de Lange ('34) describes areas, identical with those studied here in the mouse, at the base of the broad ligament in *Ctenodactylus gundi*, a rat-like rodent of Africa. He found the areas in parous females, and speaks of them as brown bodies (braune Körper). Selye and McKeown ('35) believe that the cells of these areas are the remains of the 'metrial gland.' This, they say, is probably closely related to the 'monster cells' of Minot, the 'glande endocrine myométriale' of Bouin and Ancel, and the hypertrophied endothelial cells in the vessels

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of the decidua basalis, the mesometrial wall of the uterus, and the adjacent mesometrium.

There is no question concerning the hematophagous nature of the cells of the hemosiderotic areas. There is some question concerning their origin. Since the fundamental property of reticulo-endothelial cells is phagocytosis, these cells are certainly a part of this system. Further, if they are the remains of the 'metrial gland,' then this organ belongs definitely to the reticulo-endothelial system.

The investigation outlined in this paper was concerned primarily with the uterine macrophages. Other agencies concerned in the involution of the uterus are described, and some of the possibilities of origin, function, and fate of the macrophages are discussed.

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MATERIAL AND METHODS

The animals used in all experiments were races of the common house mouse, *Mus musculus*. The original pairs were obtained from the University of Michigan breeding stock of black mice. No data are included in this paper from animals not raised under strict supervision, except for notes on several senile female white mice secured from an outside source. All females listed as virgins are of undoubted virginity. The mice were raised at normal room temperature, were not crowded and were fed on balanced laboratory rations, supplemented by adequate leafy vegetables.

A careful study was first made of virgin uteri for all stages of the oestrous cycle. This was followed by a study of uteri from pseudopregnant animals, and from gravid females, collected at various periods during gestation. Uteri from parous animals, and, finally, from senile females completed the series studied. At various periods preceding death some of the animals were subjected to intra-abdominal or subcutaneous injections of vital dyes. The ability to store these

is a characteristic property of the macrophages, often of importance in their identification. For these injections the following dyes were used: Trypan blue, trypan red, light green, carmine, brilliant vital red and India ink. For injected animals the individual dye, or combination of dyes used is discussed in the descriptions which follow.

In all cases the mice were killed by separating two of the cervical vertebrae by hand, and were dissected immediately. It was found to be of advantage to fix the entire reproductive tract attached to a piece of library card. This technique kept the uterus straight, and made it possible to get horizontal serial sections on relatively few slides. Various fixatives, dehydrating agents and stains were tried, and finally a very simple technique was adopted for all the material which was sectioned. The uteri were fixed in Gilson's solution, stained in bulk with Mayer's acid haemalum, dehydrated in ethyl alcohol and counter-stained with erythrosin. The use of a uniform technique proved very advantageous in studying the macrophages during different periods in their development. All of the illustrations have been made from slides prepared uniformly according to this simple technique.

DESCRIPTIONS OF THE UTERI

A. Primiparous uteri

1. *Early stages, from birth through the fourth day.* Extensive examination (112 specimens) of nulliparous and primigravid uteri failed to show hemosiderotic areas. Since these are characteristic of parous mice it was believed that their origin might occur during the puerperium. A study of the puerperal uterus was made in twenty-eight mice. The animals were mated, and the females were not segregated subsequently. Since the ova remain in the oviducts for about 4 days following insemination, the uterus is always non-gravid during the early part of the puerperium. When parturition seemed near, the females were observed frequently during the day. At this time several were given one or more injections of brilliant vital red, trypan blue or both. In some cases post-partum injections were also given. In all, four-

teen were injected, either before or after labor, or both. The puerperae were killed at various times up to and including 4 days post-partum. The early stages in the puerperium are from mice in which the exact time of parturition is known. For many of the later stages labor occurred during the night, and the exact hour is not known. The recorded number of hours between the birth of a litter and the death of the mother is thus an approximation. Even where parturition was observed, the timing is not accurate to the hour for an individual placental site. Frequently several hours were required for the entire litter to be born. The time recorded is the number of hours between the birth of the last of the litter, and the death of the mother. On a single slide showing two or more placental sites in horizontal section there may be a difference of some hours in the times the sites were



Fig. 1 Type of life history for primiparae killed during the puerperium; seg., segregated; sex. mat., sexual maturity; ♂, mated; cop., copulation, part. parturition.

vacated. Figure 1 gives the type of life history for these animals.

Eight uteri were removed during the first day of the puerperium, eleven during the second, five the third and four the fourth. Fifteen of these twenty-eight were sectioned serially. The number of placental sites in each uterus either equaled or was more than the number of young in the litter. The placental sites exceeded the young in number in over half of these animals. The majority, therefore, ate one or more of their young shortly after parturition. In many instances partially eaten offspring were found with the puerpera.

a. Changes at the placental site during the first day of the puerperium. The earliest stage of the puerperal uterus (no. 176) reveals the condition at the time of parturition. This mouse was killed before the last embryo of the litter had vacated the left horn. The right horn contained three placental sites with the placentae retained. One was removed

before sectioning; the others were left in place. Where the placenta was removed the separation occurred within the decidua, near the myometrium. The surface of the placental site at the base of the mesentery is ragged, and, of course, lacks the endometrium. There has been a considerable reduction in the size of the uterus. The huge blood vessels in the broad ligament are gorged with blood, probably in a static condition. They contain about the normal number of white blood cells. There are no unusually large veins, arteries or sinuses in the muscularis, or in that part of the decidua basalis which is retained.

In the figures mentioned subsequently some of the photographs of the placental sites during the early stages of the puerperium were made from multiparous uteri, studied in conjunction with the primiparous. One of these (no. 208) was removed within a few hours after parturition. All of the placentae had been expelled, and the uterine cavity was filled with the lochial fluid, which contained red and white blood cells, decidual cells and cell fragments. Figure 26, representing one of the sites in this uterus, shows the greatly distended sinuses at the uterine cavity. The thrombosed blood in one of these sinuses is separated from the lumen by little more than the endothelium. Figure 27, showing the same site at a different level, depicts portions of these thrombi separated from the wall and lying free in the uterine cavity. At the edge of this site part of a thrombosed vessel is in the process of being undermined by an invasion of the endometrium. A second site in this uterus (fig. 28) shows very clearly the distended vessels at the edge of the placental area. The appearance of the third site in this horn is seen in figure 29. A collection of leucocytes along the edge of the uterine cavity separates a mass of clotted blood from the underlying placental site. Deeper within the decidua distended and thrombosed vessels are being organized. There is a marked infiltration of leucocytes. These are brought into this area by small arteries which enter from the mesometrium. It is not easy to follow these arteries through their ultimate branches. Some end in capillaries which appear to terminate

in the placental site, with leucocytes streaming from their open ends. Whether this condition actually prevails in some of the arteries at this time, with the open ends ultimately constricting as the involution continues, can only be conjectured. The leucocytosis of labor and the puerperium has been described by Hibbard and White (1898) and many others, who find that the condition is due to an increase in the number of polymorphonuclear cells.

Williams ('31 a, '31 b) has described the disappearance in man of the placental site during the puerperium. He states that the clearing of the surface is affected by a process of exfoliation of the thrombosed and partially organized vessels. This exfoliation is produced by the extensive proliferation and ingrowth of the endometrial tissue. The placental site is undermined from its margins, and eventually is exfoliated. Williams shows polypoid masses of tissue in the process of extrusion. He finds pieces of various kinds of tissue, including fragments of obliterated vessels and endometrium, in the uterine cavity throughout the entire course of the puerperium. The uteri described here supports Williams' view on exfoliation. There is a wide difference in the time element between this phase of involution in the mouse and in man, but the process is essentially the same.

In the mouse uterus in which the placentae had been retained the absence of unusually large vessels in the decidua has been mentioned. Contrasted with this is the presence of the greatly distended sinuses in the uterus removed a few hours post-partum, and with the placentae expelled. Similarly, Williams, in a human uterus secured by a caesarean section and hysterectomy, found that the blood vessels in that part of the decidua basalis which had been retained were free from distention by blood. Conversely, in a uterus removed $\frac{3}{4}$ hour post-partum he found that the greater part of the decidua basalis was composed of large vessels distended with blood, and separated from one another by a small amount of tissue. To reconcile these observations Williams writes:

My theoretical explanation for the transformation into a well-developed placental site is as follows: Immediately

following the extrusion of the child and the placenta, the uterine musculature contracts to its maximum extent, so that the vessels throughout its wall are forcibly compressed and the relatively large, thin walled venous sinuses in the decidua basalis collapse completely. Shortly afterward, however, the uterus relaxes to a certain extent, with the result that the circulation in the thick walled arteries is partially resumed, while the thin walled veins throughout the muscularis still remain compressed. Consequently, the blood, which gains access to the uterus but finds difficulty in escaping from it, collects in the veins of the basalis and leads to their distention and engorgement. In a short time equilibrium becomes established, and, the venous contents at the placental site having already become thrombosed, the nodular structure is produced with which all pathologists and some obstetricians are familiar.

In the uterus of the rabbit during pregnancy, Barcroft and Rothschild ('32) and Barcroft, Herkel and Hill ('33) have studied the volume and rate of flow of the blood. They find that just before labor the volume is reduced to about one-third of the maximum quantity present during the latter part of gestation. They offer some possible explanations for the waxing and waning of the blood flow through the uterus shortly before birth. One possibility is that at birth the blood is being driven from the smaller vessels of the uterus in a more or less rhythmic manner. The large venous bed of the decidua acts as a sort of shock absorber, filling and emptying according to the rate of blood flow from the uterus. This insures against a venous pressure great enough to produce copious hemorrhage. These physiological observations of Barcroft and his co-workers lend support to the theoretical explanation offered by Williams for the vascular changes at the placental site during the time of labor.

Stages subsequent to the end of the first 24 hours of the puerperium show the uterine cavity filled with the lochial fluid containing many decidual cells, muscle cells and segments of newly proliferated endometrium. At the placental site erythrocytes are found free in the tissue, and pure leucocytosis is marked. There is an active ingrowth of the endometrium from the margin of the site. Toward the end

of the first day the number of leucocytes in the tissue is enormous. All incoming arteries are loaded, and in places the uterine cavity is filled with them. Masses of clotted blood are frequent among the leucocytes of the cavity. In some cases the sinuses at the edge of the placental site are so distended as to nearly fill the lumen of the uterus. These huge thrombi are broken away by the end of the second day post-partum. Figure 30 shows the exfoliation of a partially organized thrombus through ingrowth of the endometrium.

b. Changes at the placental site during the second day of the puerperium. Leucocytosis and exfoliation continue. In some cases, as shown in figure 31, an almost solid layer of leucocytes underlies the thrombosed blood which is yet to be separated from the uterine wall. Leucocytes collect around and dis sever tissue other than debris from the uterine wall. Figure 32 shows the method of removal of a group of cells which looks like regenerated endometrium. Williams has observed such loss of newly proliferated endometrium in man. The functional vessels in many cases contain about equal numbers of erythrocytes, polymorphonuclear leucocytes and lymphocytes. The lumen of the uterus is filled with cellular debris. Polymorphonuclear leucocytes, clotted blood and decidual cells predominate. Occasionally a cell identified as a mononuclear leucocyte is seen in the lochia.

Within the uterine wall some of the smaller, deeper-lying vessels of the decidua are retained. Their thrombosed blood is being organized, and figure 33 shows the invasion of connective tissue cells and leucocytes, which are surrounded by a clear area, suggesting the possibility of extracellular digestion. The retention of vessels filled with clotted blood was not seen by Williams in the puerperal uterus in man. He considers the undermining process to extend well into the muscularis, ultimately exfoliating all thrombosed and useless tissue. In effecting the exfoliation of the deeper thrombi, the endometrium grows, he explains, from the epithelial glands which lie within the myometrium, with the thrombosed vessels between them and the uterine cavity. In the mouse no glands were found proliferating at the placental area; growth of the

endometrium, as already noted, takes place only from the margin of the site. This results in a retention of some of the thrombosed vessels after the endometrium is restored.

Prenant ('26, '27) has described the post-partum transformations in the wall of certain uterine arteries in the guinea pig. The endothelium is desquamated, and the muscular fibers of the media become transformed into giant plasmodial cells which either degenerate or fall into the lumen of the artery. Histologically and functionally the adventitia takes the place of the media. These transformations were not observed in the uterus of the mouse.

Goodall ('10), in his paper on the involution of the circulatory system of the puerperal human uterus, states that the organ renews each of its arteries after a pregnancy by building the new vessel within the lumen of the old. He describes the formation of the new endothelial lining by a growth of the endothelium of the old vessel at the periphery of the clot, where a part of the lumen is not involved in the thrombosis. This idea is not supported by the mouse material. In the very early puerperium small arteries lead in from the mesentery. These are branches from the larger vessels numerous in the broad ligament, and it appears likely that these smaller arteries transform into the vessels functioning in a subsequent pregnancy.

Toward the end of the second day there is a marked ingrowth of the muscle strands from the myometrium surrounding the placental site. Figure 34 gives the general appearance of these strands, and, under higher power, figure 35 shows a portion of one next to a thrombus in the process of organization. The surrounding tissue contains great numbers of relatively large cells whose nuclei are usually eccentric in position. The cytoplasm is vacuolar, and the clear area usually contains inclusions which are stained a light pink. The inclusions are erythrocytes, ingested in their entirety or as fragments. The large cells were identified as macrophages through their property of phagocytizing the red blood cells which had been liberated into the tissue of the placental site during labor. At this time, late in the second day of the

puerperium, usually the macrophages contain only one or two red corpuscles. Frequently bits of fragmented erythrocytes constitute the only material ingested. Occasionally an amorphous mass, two or three times the size of a red blood cell, is contained in a macrophage. Often one phagocyte contains one or two whole corpuscles, and many fragments scattered throughout the cytosome.

The incoming vessels at this time show a preponderance of lymphocytes. The polymorphonuclear leucocytes are still abundant, but they no longer stream into the tissue in unusual numbers. Figure 36 shows among the macrophages a small blood vessel filled with lymphocytes and erythrocytes. Such lymphocytes are found throughout the tissue, and figure 37 shows them scattered among the macrophages. From the photomicrograph it can be seen that all sizes of nuclei are present. Examination of the tissue shows a graded series of cells ranging from the small lymphocyte, with its deeply stained circular nucleus, to the macrophage, with its larger lighter nucleus of crescentic or circular outline. This gradation of cell size is not advanced as proof that there is a genetic relationship between the two types of cells. Monocytes are not abundant in either the blood vessels or the tissue.

c. Changes at the placental site during the third and fourth days of the puerperium. At this time the restoration of the endometrium is completed, the organization of the retained thrombi continues, and the muscle strands of the nodule undergo further development. Figure 38 shows in a transverse section the position of a nodule at the base of the mesentery. Large lymph spaces bordering the nodule, and an increase in the muscularis are prominent in this specimen. Monocytes and lymphocytes are frequently found in the lymph spaces, and may enter the tissues from these as well as from the blood vessels. A horizontal section on the fourth day post-partum is shown in figure 39. The irregular mass of cells at the lumen is not an exfoliation, but a fold in the endometrium.

Examination of the macrophages shows many different stages in the digestion of the erythrocytes which have been phagocytized. Figure 40 shows a macrophage containing two undigested red blood cells. These are stained a deep pink, and were probably ingested only recently. In diameter they measure nearly 2μ more than erythrocytes contained in a nearby artery, but otherwise they look exactly like the living corpuscles. It is possible that on entering a macrophage the red cells increase in size by absorption of liquid, and that later they are broken into fragments, or massed into one large inclusion. Nearby macrophages (fig. 41) contain fragments of erythrocytes stained a paler pink, and usually each inclusion is contained in a vacuole. These cells may bear materials that have been taken in as fragments, while those shown in figure 42 contain ingested corpuscles which were taken in whole. Auer ('30) has described the formation of macrocytes and microcytes from red blood corpuscles in hanging drop cultures. Two or more erythrocytes were seen to unite, forming a macrocyte. Conversely, single erythrocytes became smaller in the cultures, each forming a microcyte. The counterparts of both macrocytes and microcytes are found here in the ingested red corpuscles within the macrophages. Occasionally the inclusion is a polymorphonuclear leucocyte, or a lymphocyte. Figure 43 gives the general appearance of macrophages containing erythrocytes in process of digestion. Cells with the ingested material in one mass are especially numerous. In these the nucleus is flattened against the periphery of the cell, giving the appearance of a signet ring, similar to fat cells. In a few cases at the end of the fourth day the cell inclusions are brown in color, lighter at first, but later darkening. Figure 44 shows these macrophages with brown inclusions. These surround a functional blood vessel, and at 4 days they are usually less abundant. Ready access to this small blood vessel and its branches may have something to do with this acceleration of the digestive process in these macrophages. Occasionally a few macrophages are found lateral to the endometrium distributed at random in the wall of the uterus.

Mitotic figures are present, but they are not abundant. One at the edge of a thrombus in process of organization is probably a fibroblast in division. In figure 45 a mitotic figure is seen among the macrophages, and figures 46 and 47 show a large macrophage whose cytosome is loaded with fragments of erythrocytes, and whose nucleus is definitely in mitosis. The two views are of the same cell at different levels. Nearly every nodule has mitotic figures, but the identification of the cell in division is not always possible. Weatherford ('33), in a study of fibrocytes and macrophages in mild acute inflammation, has not found mitosis, and from this he concludes that division is by amitosis. In the uterine nodules studied here there is no suggestion of direct division, and mitosis is seen definitely in both fibrocytes and macrophages.

Rarely a macrophage becomes much larger than its neighbors. The average size is about 16μ in diameter, but these giant cells are nearly double this size. The nucleus is proportionately large, and in one case the single nucleolus was nearly as large as the entire nucleus of a small macrophage. These over-sized phagocytes do not resemble the multinucleated giant cells described by Haythorn ('29) and others. They are not multinucleate, and simply appear to be macrophages which have enlarged beyond the usual size. There is also great irregularity in the shape of the cells. When gorged with red corpuscles many are spherical, but ovoid, triangular and irregularly shaped macrophages are numerous.

Lymphocytes and polymorphonuclear leucocytes are scattered throughout the nodule, as are mononuclear cells usually between 5 and 10μ in diameter, and with a non-granular cytosome. These may be monocytes which have migrated from the blood or lymph vessels. In a nodule it is easy to select cells ranging in size from the smallest lymphocyte or other mononuclear cells, through the larger mononuclears and small macrophages with ingested material, to the large macrophages and the giant cells. Figures 3 to 18 show such a series. Evidence of this type has been accepted by many authorities in determining the lineage of the macro-

phages. It is not included here as proof of any genetic relationship, but is intended to show the great diversity of cell types in the nodules. There is no difficulty in identifying typical lymphocytes, polymorphonuclear leucocytes, or monocytes when in the blood stream, or the macrophages when loaded with inclusions. However, it is not possible in the tissue to identify positively some of these mononuclear cells as monocytes. They might equally as well be listed as macrophages lacking inclusions, or even as fibrocytes. Further discussion on the relationship between these cell types is deferred until later.

By the end of the fourth day of the puerperium, then, the involution of the uterus is nearly completed, and at the placental site the endometrium has been restored entirely. The residual complex of liberated erythrocytes, thrombosed vessels, and necrotic tissue at the base of the mesentery has been reduced and greatly altered. In its place is a nodular structure, consisting largely of macrophages gorged with erythrocytes in various stages of digestion, muscle strands, functional blood vessels and connective tissue. These nodules, remaining at the placental sites in the dorsal wall of the uterus, may be termed placental reticulo-endothelial nodules, and are doubtless identical with the remains of the 'metrial gland,' as described by Selye and McKeown.

2. *Later stages, from the eighth through the seventy-second day.* To study the changes in the macrophages which lead to the resolution of the nodules, leaving only the brown discolorations at the mesentery, nine primiparae were killed at regular intervals after parturition. The first mouse of this series was killed 8 days after labor, and another primipara was sacrificed for every 8 day interval through 72 days. The uterus from each of these nine was sectioned. The male had been removed before parturition, and the type of life history is given in figure 2.

Some of the illustrations are taken from multiparous uteri which contain macrophages of the same age as those studied here. Four of the nine animals in this series had the same number of hemosiderotic areas as there were young in the

litter. The other five had either one or two more areas than young.

By the end of eight days the nodules have decreased to about half their size at 4 days post-partum. Connective tissue and muscle strands surround and invade each nodule, and the endometrium at the placental site is entirely normal. Nearly all of the macrophages are loaded with hemosiderin which is a golden color at this stage, sometimes very light, almost yellow, and only rarely appearing as dark brown granules. Most of the cells have one or more vacuoles which usually contain a large mass of hemosiderin, as in figures 48 and 49. Figure 50 shows a macrophage with an entirely clear vacuole. However, the digestion of red corpuscles has not progressed to the hemosiderin stage in every cell. Macrophages are abundant with the pink fragments or masses in



Fig. 2 Type of life history for primiparae killed 8 or more days post-partum; abbreviations as in figure 1.

process of digestion. Frequently one cell will contain granules of hemosiderin in one vacuole, and in another a pink inclusion in an earlier stage of digestion. Figure 51 shows this condition in a medium-sized macrophage.

At 16 days post-partum the reticulo-endothelial nodules are greatly reduced, and at their borders the connective tissue is more abundant than elsewhere in the uterine wall. The macrophages which formerly extended throughout the wall at the placental site now are found chiefly in the outer half, and extend up into the mesentery. They are much browner than in the 8-day stage, and in some cases the hemosiderin granules are very dark.

Twenty-four days after parturition the only indications of the placental sites are the hemosiderotic areas at the base of the broad ligament. In many cases the macrophages surround a blood vessel as it leaves or enters the mesentery. Sometimes three or four cells are in direct contact with one

another; others may be separated by muscle strands, connective tissue, or small blood vessels. In many the cytosome is still a golden yellow. The yellow or brown hemosiderin granules range in size from mere dots to masses several microns in diameter. They lie scattered throughout the cytosome, which also frequently contains one or more small vacuoles. Figures 19, 20 and 21 are drawings of cells at this stage. The type depicted in figure 19 is particularly common in this material. The macrophage has a large clear central vacuole with the hemosiderin in the form of fine granules located at the periphery.

For the next few weeks no marked changes occur in the hemosiderotic areas. The position of one at 48 days post-partum is shown in a horizontal section through the mesentery in figure 52. At 72 days the position of the macrophages within the parametrium at the base of the mesentery is the same as in all stages since the end of the puerperium. The cells are darker, due to the change in color of the hemosiderin, which in some is nearly black. Vacuoles still persist. Figure 53 shows a group of macrophages one of which has an especially large mass of hemosiderin in the center and the nucleus at one end of the cell. A phagocyte having two nuclei is seen in figure 54, a condition unusual in these macrophages. Figures 24 and 25 depict a group of macrophages and a large single cell at 72 days post-partum.

The presence of pigmented cells in the uterine wall of parous animals has been described by Benazzi ('28, '30 a, '30 b), Motta ('26, '27), Testa ('25) and de Lange. None of these workers, however, has outlined completely the nature, origin, function and fate of these cells. Benazzi has determined correctly that mesometrial aggregations of pigmented cells (hemosiderotic areas) are due to a previous parturition. He states that they are not found in virgins, or in primiparous mice, and he definitely relates their presence to the puerperium. Studying the African rodent *Ctenodactylus gundi*, de Lange found the hemosiderotic areas at the base of the broad ligament in parous females.

DISCUSSION

A. Origin of the macrophages in the uterus

As a basis for their opinions on the origin of the macrophages, authorities have examined principally two types of evidence: a) The presence in fixed tissues of transitional forms between the macrophages and their supposed precursors; b) the transformation of living cells in tissue cultures into macrophages. Of these the latter is no doubt the most convincing. If an individual cell could be kept under continuous observation as it transformed into a macrophage, and this process observed repeatedly, the lineage could be established definitely and unquestionably. Apparently this has not been done. The study of fixed tissues showing transitional forms between some type of cell and the macrophage cannot be said to prove the origin of the latter from the former. However, such material is often quite convincing, and in connection with the evidence from tissue cultures it may help to indicate the genetic relationships between the various cellular elements in the blood. The material examined here does not show definitely where the uterine macrophages originate. There is some slight support given to one of the theories of origin, but other possibilities are by no means ruled out. To debate extensively the question of origin would be to open a much discussed problem upon which little light is cast by the material under investigation.

Various authorities believe that the macrophages come from: 1) The general vascular endothelium, but this hypothesis is not generally accepted; 2) the specific endothelium of the liver, bone marrow, lymph glands and spleen, and this view is given a wider credence; 3) the reticulo-endothelium of special regions considered as one tissue; 4) the monocytes and lymphocytes; this theory has, perhaps, the largest following of any, and is supported in a small way by the fixed material studied in this investigation; 5) the polymorphonuclear leucocytes; and 6) the fibrocytes.

Selye and McKeown consider the macrophages which constitute a hemosiderotic area as the remains of the 'metrial gland' of the placental insertion area. They further identify

the 'metrial gland' with the scattered groups of cells found in the myometrium during the second half of gestation by various workers, and spoken of as the *glande myométriale*. In a series of brief communications Bouin and Ancel ('12 a, '12 b, '13 a, '13 b; Ancel and Bouin, '11, '13 a, '13 b) first described these cells in the rabbit, and credited them with an endocrine function of control over the mammary glands. Mercier ('12 a, '12 b, '13 a, '13 b, '13 c), Bruntz ('13), and Cuenot, Bruntz and Mercier ('13) described phagocytic cells in the uterus of the rabbit, guinea pig and mouse, also occurring during the latter part of the gestation period. These cells were termed *néphrophagocytes*, and by these men were identified with the myometrial gland of Ancel and Bouin. Mercier denies that the *nephrophagocytes* influence lactation.

Gerard ('25), writing on the myometrial gland in the mouse and rat, says that these cells elaborate special products which serve for the nutrition of the embryo. Frobose ('30) states that in the rabbit the gland is derived in part from fibrocytes, and in part from histiocytes. He speaks of the gland as resulting from a reaction of the uterus to an established pregnancy. According to him it may be a protective agency for the mother against injurious embryonic influences, or it may provide an internal secretion. Spirito ('30) believes that the myometrial gland is a part of the reticulo-endothelial system. He sees a general mobilization of this system in pregnancy, in connection with the development of the placenta.

In two critical papers on the interstitial reticulo-endothelial elements in the uterus, Albanese ('29, '30) agrees with Mercier, Spirito, and others in identifying the myometrial gland with the histocytic elements normally present in the uterus. He describes the increase in the number of these cells during pregnancy, and considers this as a reaction of the mesenchymal tissue to protein substances. In the gravid uterus these proteins have their origin in the ovarian tissue, while during the puerperium they are due to the disintegration of the necrotic cellular elements at the placental site. Albanese does not regard the myometrial gland as an organ of internal secretion.

There is considerable confusion over the identity of many of these uterine structures. If the hemosiderotic areas represent those cellular remains of the 'metrial gland' which have phagocytized erythrocytes and other cellular debris, then the identification of the organ with the reticulo-endothelial system as suggested by Albanese is certain.

B. Function of the macrophages in the uterus

Practically every paper on the macrophages refers to the part played by these cells in the digestion of the red blood corpuscles. Doan and Sabin ('26) have described phagocytosis of whole erythrocytes as well as of their fragments by circulating macrophages in the blood stream. Sandison ('31) studied the process in vivo in the rabbit's ear by means of a transparent chamber, and he gives a good description of phagocytosis and digestion of the erythrocytes. The process, as he describes it, can be followed, step by step, in a uterine nodule 3 or 4 days post-partum. The digestion of erythrocytes, then, is essentially the same in the uterus as in any situation showing hemophagocytosis.

Pianese ('29) considers the invasion of the puerperal uterus by macrophages as a response to the need for the elimination of the numberless elements of post-partum necrosis. He believes that the macrophages protect the organism from the toxic substances produced by disintegration of this organic residue. Clauser ('28) has also noted the increased activity of the reticulo-endothelial system in the uterus early in the puerperium, and he too recognizes this as a protective mechanism, effective in controlling the toxic substances formed after parturition. Hofbauer ('26) and Findley ('27) have studied the uterus in man during the puerperium, and each has demonstrated a marked increase in the number of parametrial macrophages in the puerperal organ. An increased resistance to bacterial invasion is thought to accompany this post-partum uterine reticulo-endotheliosis, and the importance of the macrophages is recognized in coping with puerperal infection.

Fluhmann ('28, '32 a, '32 b) has attempted to determine the influence of sex hormones on the uterine macrophages, and he has suggested a possible application of hormonal therapy in the treatment of pelvic inflammatory conditions. He states that in rabbits, whose uteri normally contain only a few scattered macrophages, injections of oestrin or urine of pregnant women failed to produce an increase in the number of phagocytes in the majority of cases. An injury to the uterine wall of normal rabbits resulted merely in a local mobilization of the macrophages. In animals which were given oestrin or progesterin the response to trauma was increased tremendously, with a marked rise in the number of macrophages not only at the site of injury, but throughout the whole uterus. He emphasizes their importance in inflammatory conditions, and believes that the ovarian hormones, oestrin and progesterin, increase the powers of response of the macrophages to traumatic stimuli.

Meyer ('17) has studied the intra-uterine absorption of ova in the guinea pig. His description of the giant cells which ingest erythrocytes and cell fragments indicates that the macrophages of the uterus are no doubt actively concerned in this process.

In his description of hemosiderotic areas in the uterus of *Ctenodactylus*, de Lange identifies them as glands of internal secretion. This assumption is doubtless incorrect, although it is easy to see how such an impression might be gained from the microscopic appearance of the vacuolated cells.

Selye and McKeown state that,

In addition to this nutritive function the metrial gland cells have the ability to phagocytose the debris of the placenta or of the degenerating deciduoma. These are brought in contact with them since at least part of this material is eliminated from the uterine cavity through the veins of this region. This phagocytic activity is responsible for the pigmented metrial cells, which are so frequently formed in the area of the placenta insertion after delivery.

The principal function, then, of the macrophages in the uterine wall is to reduce rapidly the post-partum nodule of

thrombosed blood, dead cells and cell remnants. Hemoglobin is the principal compound which is digested, but necrotic cells other than erythrocytes are also phagocytized. The involution of the placental site, which was started by the polymorphonuclear leucocytes and continued by the exfoliative action of the endometrium, is completed by the macrophages. In addition to their role as scavengers, the macrophages are probably important in increasing the local resistance to puerperal infections. There is no reason to believe that they function in any sense as organs of internal secretion after phagocytosis and digestion of the necrotic tissue.

C. Fate of the macrophages in the uterus

After the macrophages have ingested the necrotic cells of the nodule, and have eliminated the soluble products of digestion, they are found, gorged with hemosiderin, in the uterine wall. As the muscle of the nodule develops, and the connective tissue is renewed, the macrophages aggregate at the base of the mesentery, and remain there for an indeterminate period of time. There is no indication at 72 days post-partum of any change in the position or morphology of these cells, and the study of senile uteri indicates that they may persist throughout the life of the animal.

The general reviews on the reticulo-endothelial cells describe the so-called blockage of this system. In blockage the macrophages are supposed to become completely filled with particulate matter, and to lose their power of phagocytosis. It is not probable that such a condition ever occurs throughout the entire system. Smith ('30) states that it is difficult, if not impossible, to block the cells with injections of one vital dye so that they cannot phagocytize a second dye injected shortly thereafter. In the hemosiderotic areas, however, a local blockage seems to be complete. The macrophages are effectively removed from further functioning, and the hemosiderin is stored at the base of the mesentery for a considerable time.

In the lung Permar ('20) describes the migration of blocked macrophages to the hilus of the lymph nodes, where they re-

main quiescent for long periods. Permar believes that the blocked macrophages of the lung may become reactivated, and migrate to a site of injury where they resume phagocytosis. There is no indication of such reactivation of the cells in the hemosiderotic areas of the uterus. Maximow (Cowdry, '28) states that the macrophages may transfer their particulate matter to other cells. He also describes the degeneration and disintegration of macrophages, in which the inclusions are set free. These may be carried away by the lymph or blood, or taken up by other phagocytes. The uterine macrophages show no signs of disintegration, and at no time were granules of hemosiderin found free in the wall of the uterus.

Further experiments on primiparous mice which have been segregated for a year or more after parturition are needed to show definitely the length of time that the macrophages persist in the parametrium. It is also desirable to examine the cells in senile females whose sexual history is definitely known. Such experiments have been started, and it is hoped that a later paper may report positively on the ultimate fate of the uterine macrophages. For the present they are known to remain intact at the base of the mesentery for some months, and it is believed that they may remain in this position for a year or more, possibly throughout the life of the animal, or about 2 years.

CONCLUSIONS

To summarize the involution of the uterus in the mouse, then, we may say that it is accomplished through the concurrence of at least three major agencies: 1) The effect of polymorphonuclear leucocytes in digesting and eliminating cellular debris during the first 2 days of the puerperium; 2) the exfoliation of polypoid masses of thrombosed vessels and other necrotic residuum through undermining by the endometrium during the first few days post-partum; and 3) the phagocytosis and digestion by the macrophages of erythrocytes and other cells or cell remnants after the second day following parturition. These agencies rapidly transform the injured and disorganized placental site into the meso-

metrial reticulo-endothelial nodule characteristic of the puerperium after the first 2 or 3 days.

The macrophages, gorged with hemosiderin as a result of the digestion of red blood cells, remain intact in aggregations at the base of the mesentery for several months, and possibly throughout the life of the animal. These make up the small brown areas seen macroscopically on the dorsal surface of the uterus, and spoken of as the hemosiderotic areas. The exact origin of the cells of these aggregations is undetermined, but the possibility is recognized that they may come in part from the scattered macrophages present normally in the uterus, and in part from monocytes or lymphocytes which migrate from the blood stream.

LITERATURE CITED

- ALBANESE, A. 1929 Sul significato degli elementi interstiziali dell'utero e sui rapporti col reticolo-endotelio. *Riv. ital. di ginec.*, vol. 9, pp. 441-462.
- 1930 La reazione istogena di difesa dell'utero. *Arch. di ostet. e ginec.*, vol. 17, pp. 523-535.
- ANCEL, P. AND P. BOUIN 1911 Sur l'existence d'une glande myométriale endocrine chez la lapine gestante. *Compt. rend. Assoc. des Anat.*, Treizième Réunion, Paris, pp. 97-103.
- 1913 a Sur les soi-disant néphrophagocytes utérins et la signification des cellules myométriales. *Compt. rend. Soc. de biol.*, T. 74, pp. 352-354.
- 1913 b La méthode des injections physiologiques et la détermination des cellules excrétrices. *Compt. rend. Soc. de biol.*, T. 74, pp. 1209-1211.
- AUER, J. 1930 Formation of macrocytes and microcytes from normal red blood corpuscles. *Proc. Soc. Exper. Biol. and Med.*, vol. 27, pp. 620-622.
- BARCROFT, J. AND P. ROTHSCHILD 1932 The volume of blood in the uterus during pregnancy. *J. Physiol.*, vol. 76, pp. 447-459.
- BARCROFT, J., W. HERKEL AND S. HILL 1933 The rate of blood flow and gaseous metabolism of the uterus during pregnancy. *J. Physiol.*, vol. 77, pp. 194-206.
- BENAZZI, M. 1928 Sulla esistenza di particolari cellule interstiziali nel connettivo dell'utero di topo. *Atti. d. r. Accad. naz. d. Lincei*, vol. 7, pp. 259-261.
- 1930 a Sull'origine del pigmento nell'utero di *Mus musculus*. *Boll. d. Soc. ital. di biol. sperim.*, vol. 5, pp. 336-338.
- 1930 b Contributo alla istofisiologia dell'utero di Mammiferi con particolare riguardo alla conoscenza delle cellule pigmentate. *Arch. ital. di anat. e di embriol.*, vol. 28, pp. 163-210.
- BOUIN, P. AND P. ANCEL 1912 a Sur l'évolution de la glande mammaire pendant la gestation. Déterminisme de la phase glandulaire gravidique. *Compt. rend. Soc. de biol.*, T. 72, pp. 129-131.
- 1912 b A propos de la glande myométriale. *Compt. rend. Soc. de biol.*, T. 73, pp. 637-639.
- 1913 a Sur les cellules du myométrium qui prennent le carmin des injections physiologiques. *Compt. rend. Soc. de biol.*, T. 74, pp. 728-729.
- 1913 b Détermination des cellules excrétrices par le procédé des injections physiologiques de matières colorantes. *Compt. rend. Soc. de biol.*, T. 74, pp. 890-892.

- BRUNTZ, L. 1913 A propos des néphrocytes et des néphrophagocytes. *Compt. rend. Soc. de biol.*, T. 74, pp. 643-645.
- CLAUSER, F. 1928 Sull'attività funzionale dell'apparato reticolo-endoteliale nello stato puerperale. *Ann. di ostet. e ginec.*, vol. 50, pp. 411-424.
- COWDEY, E. V. 1928 *Special cytology*. Paul B. Hoeber, Inc., New York.
- CUÉNOT, L., L. BRUNTZ AND L. MERCIER 1913 Examen des critiques faites a la méthode des injections physiologiques. *Compt. rend. Soc. de biol.*, T. 74, pp. 1124-1125.
- DOAN, C. A. AND F. R. SABIN 1926 Normal and pathological fragmentation of red blood cells; the phagocytosis of these fragments by desquamated endothelial cells of the blood stream; the correlation of the peroxidase reaction with phagocytosis in mononuclear cells. *J. Exp. Med.*, vol. 43, pp. 839-850.
- FINDLEY, P. 1927 The biologic defense in puerperal infection. *Am. J. Obst. and Gynec.*, vol. 13, pp. 514-517.
- FLUHMANN, C. F. 1928 The reticulo-endothelial cells of the uterus: An experimental study. *Am. J. Obst. and Gynec.*, vol. 15, pp. 783-796.
- 1932 a Influence of sex hormones on the occurrence of tissue macrophages in the rabbit's uterus. *Proc. Soc. Exp. Biol. and Med.*, vol. 29, pp. 1027-1028.
- 1932 b The influence of sex hormones on the reticulo-endothelial cells of the uterus and a possible application to the treatment of pelvic inflammatory conditions. *Am. J. Obst. and Gynec.*, vol. 15, pp. 654-667.
- FROBOSE, H. 1930 Beiträge zur mikroskopischen Anatomie des Kaninchenuterus. I. Ueber einkernige Riesenzellen in der Obplacenta und über die "Glande myométriale endocrine." *Jahrb. f. Morphol. u. mikr. Anat. Abt. II: Ztschr. f. mikr.-anat. Forsch.*, Bd. 23, S. 121-168.
- GERARD, P. 1925 Sur la glande myométriale de la Souris et du Rat. *Compt. rend. Soc. de biol.*, T. 93, pp. 457-459.
- GOODALL, J. R. 1910 The involution of the puerperal uterus, and, more particularly, the involution of its circulatory system, vitreous hypertrophy and vitreous degeneration of elastic tissue. *Royal Victoria Hosp., Montreal, Studies.*, vol. 2, no. 3, pp. 1-76.
- HAYTHORN, S. R. 1929 Multinucleated giant cells with particular reference to the foreign body giant cell. *Arch. Path.*, vol. 7, pp. 651-713.
- HIBBARD, C. M. AND F. W. WHITE 1898 The leucocytosis of labor and the puerperium. *J. Exp. Med.*, vol. 3, pp. 639-646.
- HOFBAUER, J. 1926 The defensive mechanism of the parametrium during pregnancy and labor. *Bull. Johns Hopkins Hosp.*, vol. 38, pp. 255-272.
- DE LANGE, D. 1934 Beobachtungen an puerperalen und schwangeren Uteri von *Ctenodactylus gundi*. *Jahrb. f. Morphol. u. mikr. Anat. Abt. II: Ztschr. f. mikr.-anat. Forsch.*, Bd. 36, S. 488-496.
- MERCIER, L. 1912 a Sur l'existence de néphrophagocytes dans le muscle utérin de femelles de Mammifères en gestation. *Compt. rend. Soc. de biol.*, T. 72, pp. 212-214.
- 1912 b Recherches sur les néphrophagocytes de l'utérus gravide chez la lapine. *Compt. rend. Soc. de biol.*, T. 73, pp. 534-536.
- 1913 a A propos des néphrophagocytes de l'utérus de la lapine gestante. *Compt. rend. Soc. de biol.*, T. 74, pp. 165-166.
- 1913 b A propos du déterminisme de la sécrétion mammaire chez la lapine. *Compt. rend. Soc. de biol.*, T. 74, pp. 646-648.
- 1913 c Etat de nos connaissances sur le déterminisme de l'apparition du lait chez la lapine gestante. *Compt. rend. Soc. de biol.*, T. 74, p. 887.
- MEYER, A. W. 1917 Intra-uterine absorption of ova. *Anat. Rec.*, vol. 12, pp. 293-307.
- MOTTA, G. 1926 Sul metabolismo del ferro nell'utero. *Boll. d. Soc. ital. biol. di sperim.*, vol. 1, pp. 242-244.
- 1927 Gli elementi reticolo-endoteliali dell'utero. *Haematologica*, vol. 8, pp. 91-105.

- PERMAR, H. H. 1920 The migration and fate of the mononuclear phagocyte of the lung. *J. M. Research*, vol. 42, pp. 209-225.
- PIANESE, F. 1929 Il sistema reticolo-istiocitario o sistema di Goldmann nell'utero gravido e puerperale della cavia. *Arch. di ostet. e ginec.*, vol. 16, pp. 203-222.
- PRENANT, A. 1926 Sur les transformations de la paroi de certaines artères dans l'utérus du cobaye après parturition. *Compt. rend. Assoc. des Anat.*, Vingt et unième Réunion, Liège, pp. 495-500.
- 1927 Recherches sur les transformations de la paroi de certaines artères dans l'utérus du cobaye après parturition. *Arch. d'anat., d'histol., et d'embryol.*, T. 7, pp. 163-196.
- SANDISON, J. C. 1931 Observations on the circulating blood cells, adventitial (Rouget) and muscle cells, endothelium, and macrophages in the transparent chamber of the rabbit's ear. *Anat. Rec.*, vol. 50, pp. 355-379.
- SELYE, H. AND T. McKEOWN 1935 Studies on the physiology of the maternal placenta in the rat. *Proc. Roy. Soc., London, B.*, vol. 119, pp. 1-31.
- SMITH, H. P. 1930 Studies on vital staining. III. The simultaneous ingestion of two dyestuffs by phagocytes. The question of "blockade of the reticulo-endothelial system." *J. Exp. Med.*, vol. 51, pp. 395-409.
- SPIRITO, F. 1930 Il sistema reticolo-istiocitario nel campo ostetrico-ginecologico. *Gazz. Sanit. (Milano)*, vol. 3, pp. 9-10. Quoted from *Biol. Abst.*, 1933, vol. 7, p. 1985.
- TESTA, M. 1925 Contributo alla etiopatogenesi della gravidanza ectopica e alla conoscenza del meccanismo di rottura della tuba gravida. *Arch. di ostet. e ginec.*, vol. 12, pp. 49-72.
- WEATHERFORD, H. L. 1933 Chondriosomal changes in connective-tissue cells in the initial stages of acute inflammation. *Ztschr. f. wissenschaft. Biol.*, Abt. B.: *Ztschr. f. Zellforsch. u. mikr. Anat.*, Bd. 17, S. 518-541.
- WILLIAMS, J. W. 1931 a Disappearance of the placental site during the puerperium. *J. Am. Med. Assoc.*, vol. 97, pp. 523-529.
- 1931 b Regeneration of the uterine mucosa after delivery, with especial reference to the placental site. *Am. J. Obst. and Gynec.*, vol. 22, pp. 664-696.

PLATE 1

EXPLANATION OF FIGURES

All outlines were made by means of a camera lucida. Types of cells found in a mesometrial reticulo-endothelial nodule.

- 3 Lymphocyte.
4 to 9 Mononuclear leucocytes.
10 to 18 Macrophages.

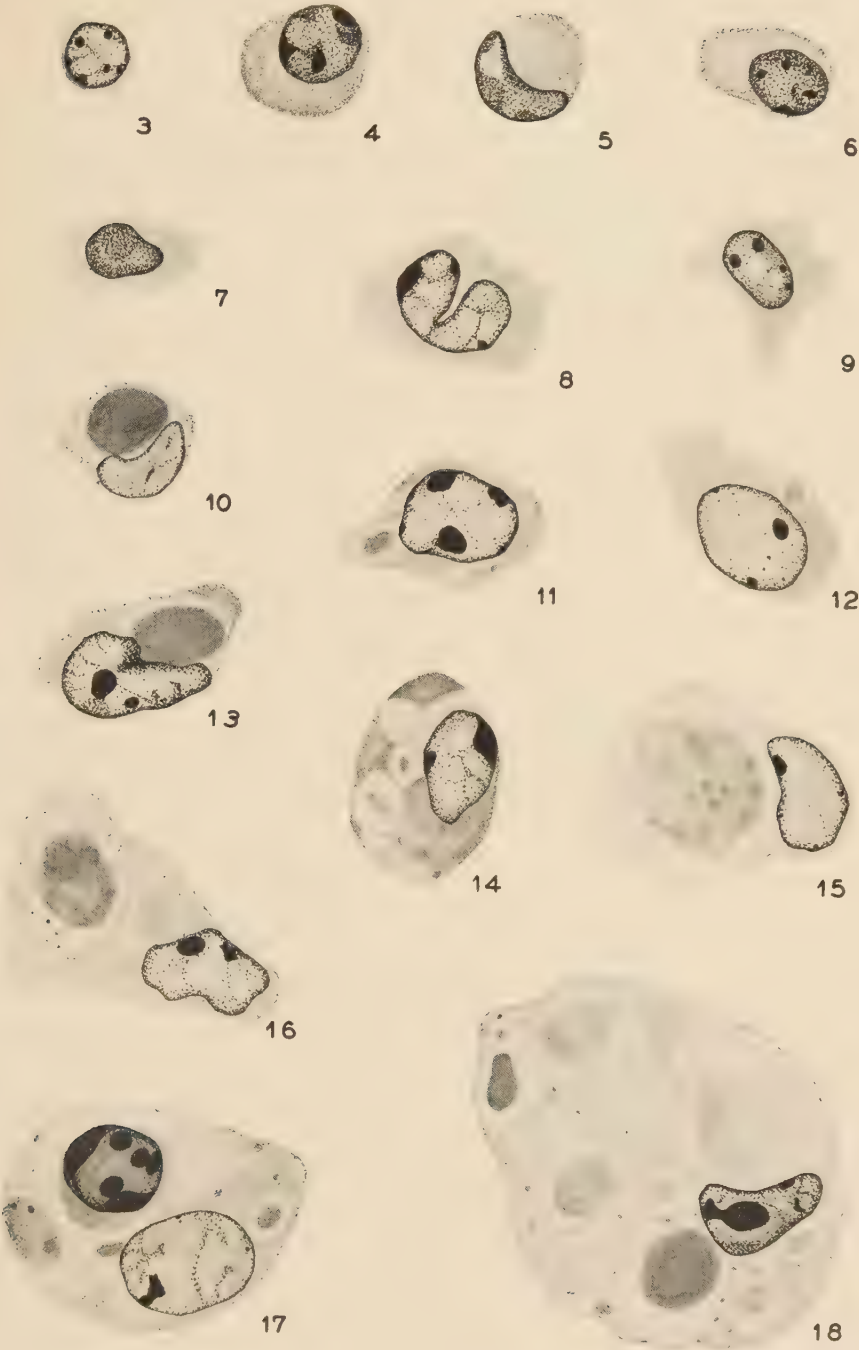


PLATE 2

EXPLANATION OF FIGURES

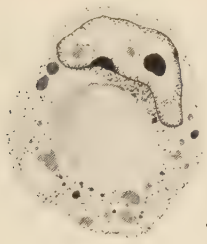
All outlines were made by means of a camera lucida.

19 to 21 Macrophages showing the appearance of the cells in a hemosiderotic area at 24 days post-partum.

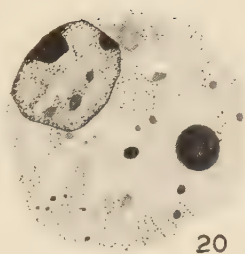
22 Lymphocyte from the uterine wall at 24 days post-partum.

23 Polymorphonuclear leucocyte from the uterine wall at 24 days post-partum.

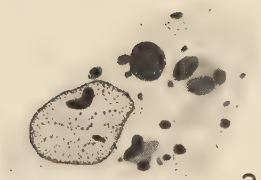
24 and 25 Macrophages showing the appearance of the cells in a hemosiderotic area at 72 days post-partum.



19



20



21



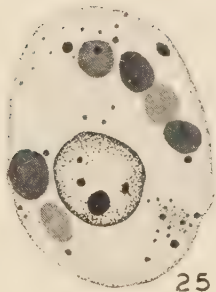
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PLATE 3

EXPLANATION OF FIGURES

Photographs of sections, placental site of mouse.

26 Edge of the placental site a few hours post-partum, showing distended sinuses at the uterine cavity.

27 Margin of the same site shown in figure 26, showing parts of a thrombus free in the uterine cavity.

28 Edge of another placental site a few hours post-partum, showing the distended vessels at the edge of the placental area.

29 Edge of a third placental site a few hours post-partum, showing thrombus being broken up and exfoliated into the uterine cavity through the action of polymorphonuclear leucocytes.

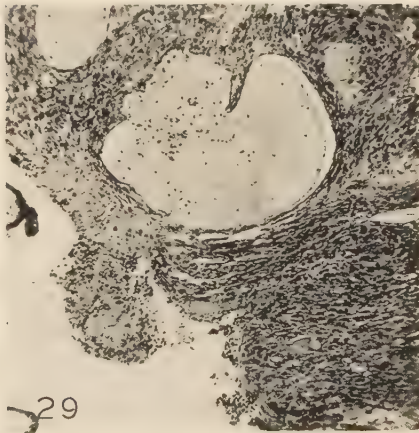
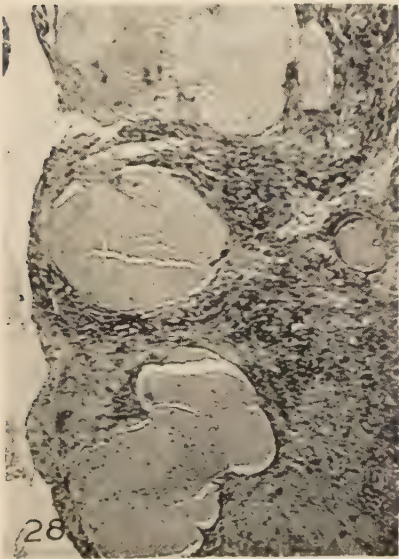
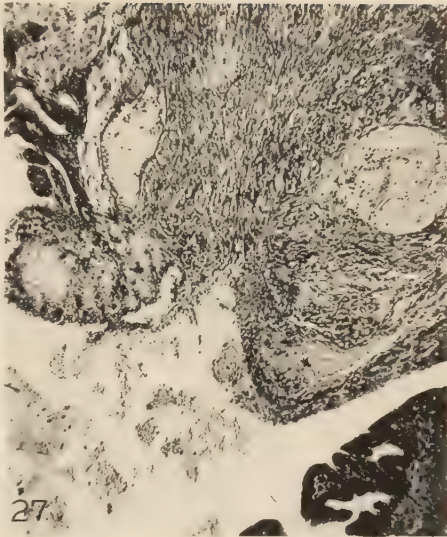
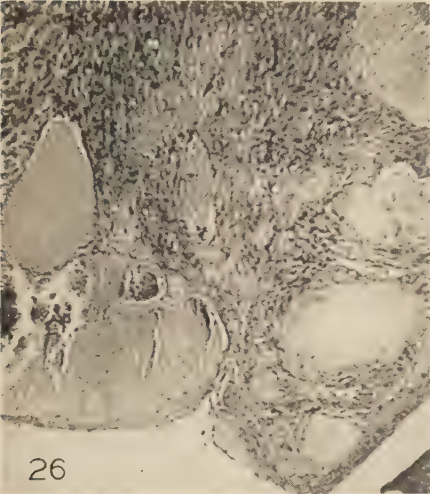


PLATE 4

EXPLANATION OF FIGURES

Photographs of sections, placental site of mouse.

30 Edge of a placental site toward the end of the first day post-partum, showing exfoliation of a partially organized thrombus through the ingrowth of endometrium.

31 Edge of a placental site during the second day post-partum, showing thrombus separated from the uterine wall by an almost solid layer of polymorphonuclear leucocytes.

32 Edge of a placental site during the second day post-partum, showing the exfoliation of a segment of regenerated endometrium by polymorphonuclear leucocytes.

33 Thrombosed vessel retained within the uterine wall, and showing organization through the invasion of fibrocytes and leucocytes.

34 Placental area showing ingrowth of muscle strands.

35 Thrombus at the placental site in process of organization, and showing a prominent strand of muscle tissue.

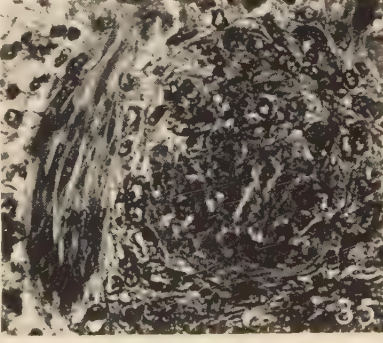
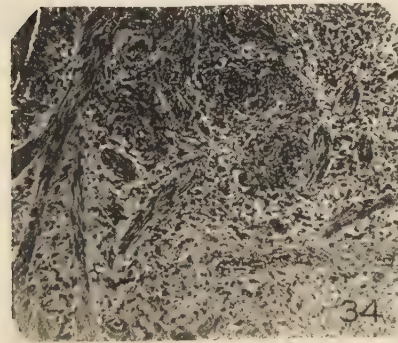
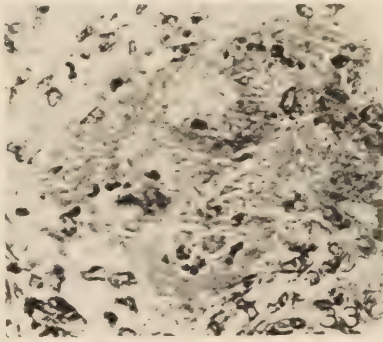
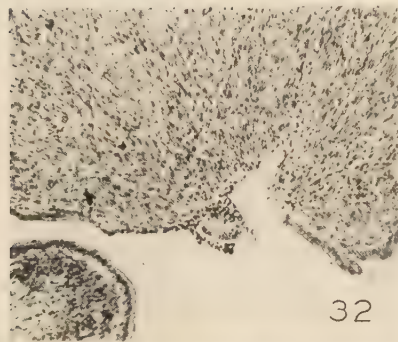
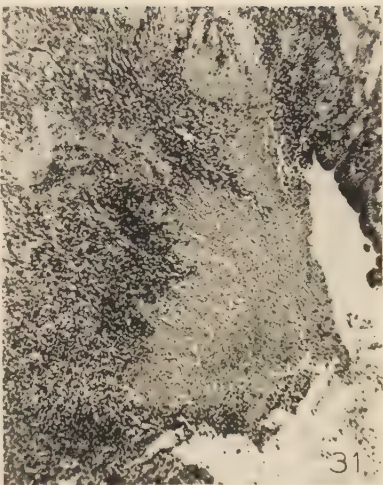
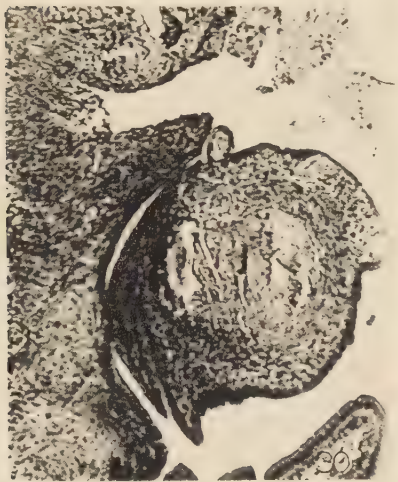


PLATE 5

EXPLANATION OF FIGURES

Photographs of sections, puerperal uterus of mouse.

36 Placental site, second day post-partum, showing among the macrophages a small blood vessel filled with lymphocytes and erythrocytes.

37 Placental site at the end of the second day post-partum, showing large numbers of lymphocytes scattered among the macrophages.

38 Transverse section of uterus 4 days post-partum, showing a reticulo-endothelial nodule at the base of the mesentery.

39 Horizontal section through a nodule 4 days post-partum.

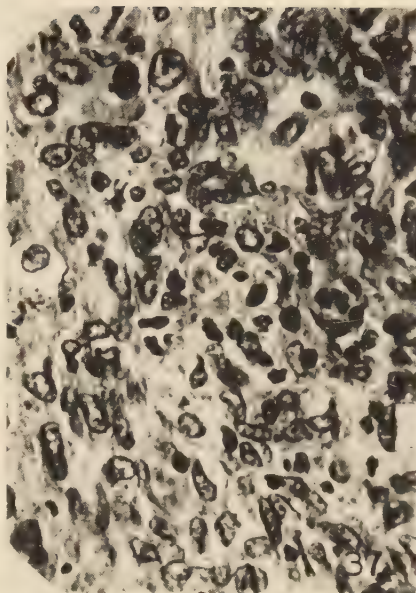
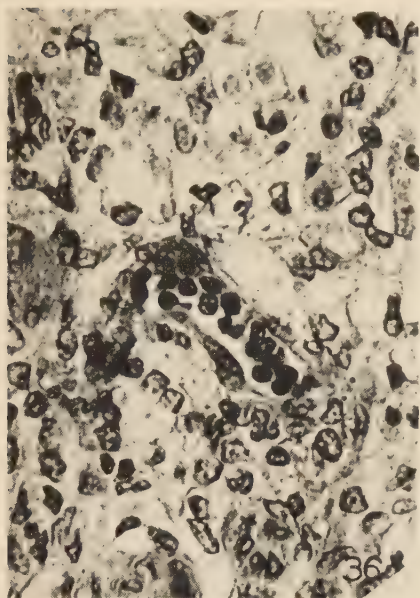


PLATE 6

EXPLANATION OF FIGURES

Photographs of sections, reticulo-endothelial nodule 4 days post-partum.

- 40 A macrophage showing two undigested erythrocytes which have been phagocytized.
- 41 Macrophages containing fragments of erythrocytes, each fragment enclosed in a vacuole.
- 42 A macrophage showing two large masses of ingested material contained in a vacuole.
- 43 General appearance of a field of macrophages showing erythrocytes in various stages of digestion.
- 44 General appearance of a field of macrophages in which the inclusions are turning brown.
- 45 Mitotic figure among the macrophages, probably a macrophage in division.

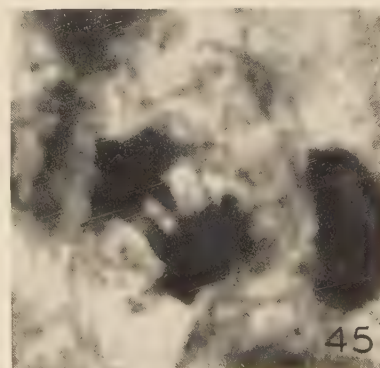
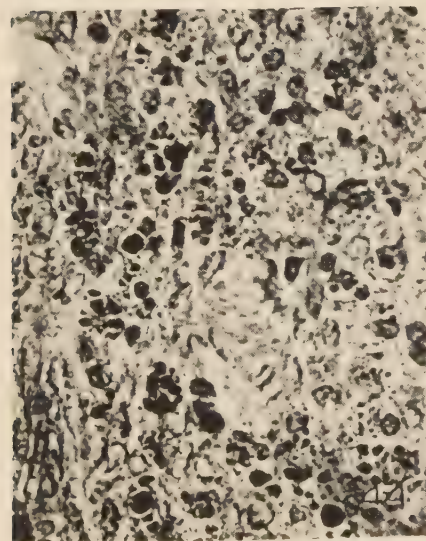
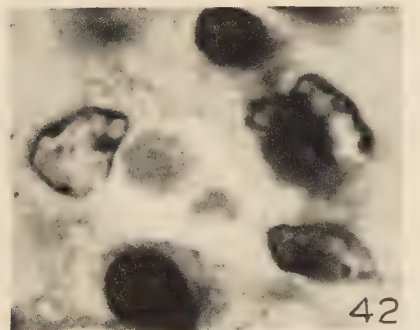
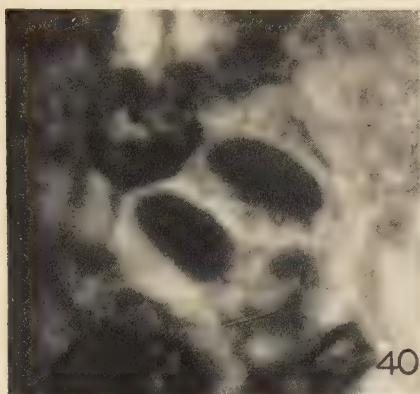


PLATE 7

EXPLANATION OF FIGURES

Photographs of sections, reticulo-endothelial nodule.

- 46 Large macrophage in mitosis, showing the cytosome filled with fragments of erythrocytes. 4 days post-partum.
- 47 Same cell as in figure 46 at a different level, showing the mitotic figure.
- 48 A macrophage at 8 days post-partum, showing a mass of hemosiderin in the central vacuole, and granules of hemosiderin at the periphery.
- 49 Macrophages at 8 days post-partum.

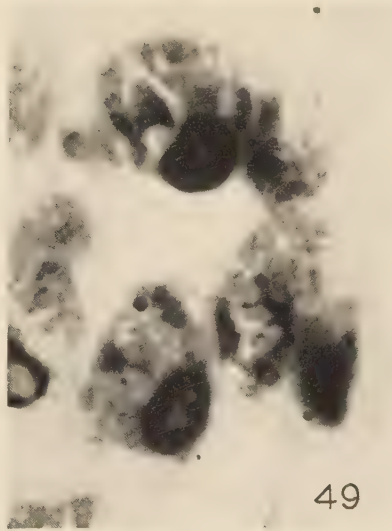
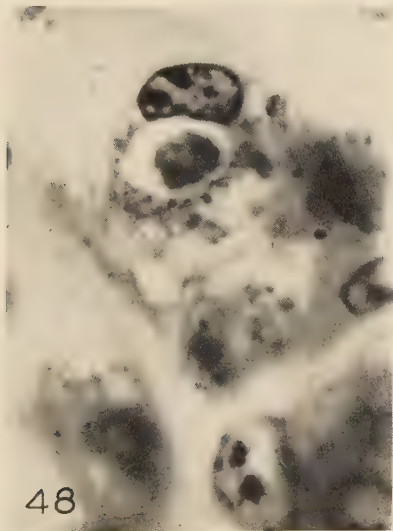
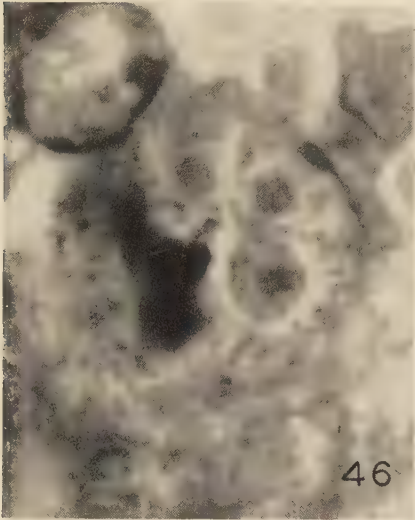


PLATE 8

EXPLANATION OF FIGURES

Photographs of sections, hemosiderotic areas following the puerperium.

- 50 Two macrophages at 8 days post-partum, with one showing a clear vacuole.
- 51 Macrophages at 8 days post-partum, the one in the center showing granules of hemosiderin in one vacuole, and in a second vacuole a mass of material in an earlier stage of digestion.
- 52 Horizontal section through the uterine wall at the base of the mesentery 48 days post-partum, showing the aggregation of macrophages which make up one hemosiderotic area.
- 53 Macrophages at 72 days post-partum. The upper cell contains an especially large mass of hemosiderin, centrally located.
- 54 Macrophages at 72 days post-partum, showing an elongated cell having two nuclei.

